

## **AMENDMENTS TO THE CLAIMS**

Claims 1-29 are pending in the application. By this Amendment, claim 20 is amended and claims 25-29 are added. Claims 1-19 and 21-24 are withdrawn from further consideration.

1. (Withdrawn) A detection kit comprising:

a measurement plate having a plate body that has a bottomed well wherein a sample is injected and a primary antibody that is solid-phased on a surface of the well and recognizes a frog vitellogenin;

a standard frog vitellogenin that is injected in the well where the primary antibody is solid-phased; and

a secondary antibody that is injected in the well where the sample or standard frog vitellogenin is injected to recognize the frog vitellogenin.

2. (Withdrawn) The detection kit according to claim 1, wherein the sample is a frog blood plasma or blood serum.

3. (Withdrawn) The detection kit according to claim 1, wherein the secondary antibody is labeled with a labeling compound.

4. (Withdrawn) The detection kit according to claim 1, wherein the primary antibody is adsorbed on the surface of the well and the surface of the well is blocked with a blocking agent.

5. (Withdrawn) A detection kit comprising:

a first plate that has a bottomed well where a sample and an antibody are injected and mixed, the antibody recognizing a frog vitellogenin and labeled with a labeling compound;

a second plate having a bottomed well in which a mixture liquid of the sample and antibody is injected; and

a standard frog vitellogenin that is solid-phased as an antigen on a surface of the well of the second plate.

6. (Withdrawn) The detection kit according to claim 5, wherein the sample is a frog blood plasma or blood serum.

7. (Withdrawn) The detection kit according to claim 5, wherein the antigen is solid-phased on the surface of the well of the second plate and blocked with a blocking agent.

8. (Withdrawn) A measurement plate comprising:

a plate body that has a bottomed well wherein a sample is injected; and

a primary antibody that is solid-phased on a surface of the well and recognizes a frog vitellogenin.

9. (Withdrawn) A measurement plate comprising:

a plate body that has a bottomed well where a mixture of a sample and an antibody is injected, the antibody recognizing a frog vitellogenin and labeled with a labeling compound; and

a frog vitellogenin that is solid-phased as an antigen on a surface of the well of the plate.

10. (Withdrawn) A detection method to detect a frog vitellogenin with a detection kit according to claims 1-6 or 7.

11. (Withdrawn) A detection method comprising the steps of:

reacting a sample and a primary antibody that recognizes a vitellogenin contained in the sample; and

reacting a complex and a secondary antibody, the complex compounded of the vitellogenin and the primary antibody, the secondary antibody recognizing the vitellogenin.

12. (Withdrawn) The detection method according to claim 11, wherein the secondary antibody is labeled with a labeling compound.

13. (Withdrawn) The detection method according to claim 11 or 12, further comprising the step of:

directly or indirectly reacting the secondary antibody bonded with the complex and a chromogenic reagent to measure based on a coloring reaction thereof an amount of vitellogenin in the test body.

14. (Withdrawn) A detection method comprising the steps of:

reacting a sample and a primary antibody that is labeled with a labeling compound and recognizes a vitellogenin contained in the sample to obtain a complex; and

competitively reacting the complex and a vitellogenin.

15. (Withdrawn) The detection method according to claim 14 further comprising the step of:

reacting a reaction product obtained according to the competitive reaction and a chromogenic reagent to measure based on a coloring reaction therebetween an amount of the vitellogenin in the sample.

16. (Withdrawn) An evaluation method comprising the steps of:

reacting a sample and a primary antibody that recognizes a vitellogenin contained in the sample;

reacting a secondary antibody that is labeled with a labeling compound and recognizes the vitellogenin with a complex of the vitellogenin contained in the sample and the primary antibody;

reacting a label in the secondary antibody bonded to the complex and a chromogenic reagent to measure an stained amount; and

calculating an amount of the vitellogenin from the stained amount to evaluate based on the amount of the vitellogenin.

17. (Withdrawn) The environment evaluation method according to claim 16, wherein the sample is a frog blood plasma or blood serum.

18. (Withdrawn) An evaluation method comprising the steps of:

reacting a sample and an antibody that is labeled with a labeling compound and recognizes a frog vitellogenin contained in the sample to obtain a complex;

causing the complex and vitellogenin to competitively react; and

reacting a reaction product obtained according to the competitive reaction and a chromogenic reagent, calculating based on a coloring reaction thereof an amount of vitellogenin in the test body to evaluate based on the amount of the vitellogenin.

19. (Withdrawn) The evaluation method according to claim 18, wherein the sample is a frog blood plasma or blood serum.

20. (Currently Amended) A polyclonal antibody ~~of~~ specific to a frog vitellogenin, produced by the processes of:

immunizing a mammal with a frog vitellogenin as an antigen;

~~sampling an anti blood serum from the immunized mammal; and~~

~~isolating as an IgG from the anti blood serum~~

collecting an antiserum from the immunized mammal;

preparing IgG from the antiserum; and

purifying the IgG by an adsorption purification column coupled with blood serum proteins of a male frog and an affinity purification column coupled with a frog vitellogenin.

21. (Withdrawn) A manufacturing method of a frog vitellogenin antibody, comprising the steps of:

obtaining an IgG from an anti-blood serum sampled after a mammal is immunized with a frog vitellogenin as an antigen; and

purifying the IgG with an affinity column.

22. (Withdrawn) The manufacturing method of a frog vitellogenin antibody according to claim 21, wherein the affinity column is bonded with a male frog serum protein.

23. (Withdrawn) The manufacturing method of a frog vitellogenin antibody according to claim 22, wherein the affinity column is bonded with a frog vitellogenin.

24. (Withdrawn) An evaluation method comprising the steps of:

cultivating a hepatocyte due to an amphibian;

administering a sample to the hepatocyte; and

detecting a response to the sample of the cultivated hepatocyte.

25. (NEW) The polyclonal antibody according to claim 20,  
wherein antibodies to proteins in the blood serum of the male frog are  
eliminated by the process of the adsorption purification.

26. (NEW) The polyclonal antibody according to claim 20,  
wherein antibodies to the frog vitellogenin are bonded to the affinity  
purification column by the process of the affinity purification.

27. (NEW) The polyclonal antibody according to claim 20,  
wherein the process of the affinity purification is done next to the process  
of the adsorption purification.

28. (NEW) The polyclonal antibody according to claim 20,  
wherein the frog vitellogenin as antigen is purified by chromatography from  
blood serum of an male frog in which vitellogenin synthesis is induced.

29. (NEW) The polyclonal antibody according to claim 20,  
wherein the frog vitellogenin used in the process of the affinity purification is purified by  
column chromatography from blood serum of an male frog in which vitellogenin  
synthesis is induced.